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Objective and quantitative evaluation of motor function in a monkey model of Parkinson's disease

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ABSTRACT

Monkeys treated with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) are currently the best animal model for Parkinson's disease (PD) and have been widely used for physiological and pharmacological investigations. However, objective and quantitative assessments have not been established for grading their motor behaviors. In order to develop a method for an unbiased evaluation, we performed a video-based assessment, used qualitative rating scales, and carried out an *in vivo* investigation of dopamine (DA) transporter binding in systemically MPTP-treated monkeys. The video-based analysis of spontaneous movement clearly demonstrated a significant correlation with the qualitative rating score. The assessment of DA transporter (DAT) function by [^{11}C]-CFT-PET showed that, when compared with normal animals, the MPTP-treated animals exhibited decreased CFT binding in the bilateral striatum, particularly in the dorsal part in the putamen and caudate. Among the MPTP-treated monkeys, an unbiased PET analysis revealed a significant correlation between CFT binding in the midbrain and qualitative rating scores or the amount of spontaneous movements. These results indicate that a video-based analysis can be a reliable tool for an objective and quantitative evaluation of motor dysfunction of MPTP-treated monkeys, and furthermore, that DAT function in the midbrain may also be important for the evaluation.

1. Introduction

In rodents, non-human primates, and humans, systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) causes the selective loss of dopamine (DA) neurons in the substantia nigra (SN), as seen in patients with Parkinson's disease (PD). The treatment of monkeys with MPTP has become the most successful primate model of human neurodegenerative disease. This treatment evokes a persistent syndrome in the monkey and produces virtually all of the cardinal behavioral, biochemical, and histological changes that occur within the DA system in PD (Andersson et al., 2006; Burns et al., 1983). Furthermore, most of the current anti-parkinsonism therapies were tested for efficacy and approved based on this model. Thus, MPTP-treated animals have been contributing to physiological and pharmacological investigations as PD models (Jenner, 2003).

A number of qualitative rating scales have been employed to evaluate the neurological functions of MPTP-treated monkeys. These scales, however, are functions of multiple variables, and the impact on each variable differs among the scales (Imbert et al., 2000). Therefore, more objective evaluation methods are needed for an accurate comparison of the effects of anti-parkinsonism therapies. Previous studies have used a video-based analysis system as a qualitative assessment of behavioral evaluations of rodents and non-human primates (Chassain et al., 2001; Liu et al., 2009; Togasaki et al., 2005). This type of system provides qualitative interpretations of movement, or the measurement of specific actions, as well as a quantitative assessment. Thus, the first aim of this study was to validate a video-based movement analysis system by comparing its results with behavioral evaluation using a qualitative rating scale.

An objective evaluation for indicating the function of the DA system can be also obtained using neuroimaging techniques. Specifically, positron emission tomography (PET) using 6-[^{18}F]-fluoro-3,4-dihydroxy-L-phenylalanine ([^{18}F]-F-DOPA), [^{11}C]-2β-carbomethoxy-3β-(4-fluorophenyl)-tropane ([^{11}C]-CFT), and [^{11}C]-raclopride has been clinically used to evaluate DA synthesis capacity, DA transporter (DAT), and DA receptors, respectively. Several PET studies in humans (Bruck et al., 2009; Nurmi et al., 2003; Rinne et al., 2001) and monkeys (Oiwa et al., 2003; Wullner et al., 1994) have demonstrated a behavior-related decrease in DA

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synthesis or DAT density in the striatum. However, the behavioral evaluations that were used were not fully established as objective, and the anatomical focus of these studies was the striatum, where DA and DAT are most abundant. Thus, the second aim of this study was to investigate a correlation between the whole brain PET analysis and the behavior evaluations. In particular, we focused on DAT as an indicator of DA function, because it has been reported that CFT-PET is more sensitive than F-DOPA-PET in detecting DA hypofunction (Forsback et al., 2004). The DAT is a protein localized on the presynaptic membrane of DA neuron terminals; it clears DA from the interstitial space back into presynaptic elements by a process of facilitated diffusion (Kuhar et al., 1990). In addition, several studies have shown that a substantial amount of DAT also localizes in the somatodendritic membrane of DA neurons (Cheramy et al., 1981; Cobb and Abercrombie, 2002; Timmerman and Abercrombie, 1996) where it modulates local DA transmission within the SN (Vandecasteele et al., 2008) and thus may have a distinct role from that of nigro-striatal DA (Cheramy et al., 1981).

To accomplish these aims, we evaluated the neurological functions of MPTP-treated monkeys using a qualitative rating scale and video-based analysis system. We then performed an unbiased voxel-based analysis of the whole brain using [^{11}C]-CFT-PET.

2. Methods

2.1. Animals

Adult (4 y.o.) cynomolgus monkeys (*Macaca fascicularis*), weighing 3.3–4.0 kg, were provided by Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan for this study. Animals in the MPTP-treated group ($n = 13$) were given intravenous injections of MPTP HCl (0.4 mg/kg as free base, Sigma–Aldrich) twice a week until persistent parkinsonian behavioral disturbances, such as tremor, bradykinesia, and impaired balance, became evident. The animals received an average of 15.6 MPTP administrations, and those that presented stable parkinsonism for over 12 weeks were used for the experiments. We also used normal monkeys for the [^{11}C]-CFT-PET study ($n = 5$) and for behavioral estimation ($n = 8$). Monkeys were cared for and handled according to the Guidelines for Animal Experiments of Kyoto University and the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

2.2. Qualitative rating scale

The behavior of the animals was evaluated according to a rating scale for monkey PD models (Takagi et al., 2005). This scale rates nine items: alertness (0–2); head checking movement (0–2); eye blinking and movement (0–2); posture (0–3); balance (0–3); motility at rest (0–3); reactive motility to external stimuli (0–3); walking (0–3); and tremor (0–3). Normal and minimum score is 0, and maximum total score is 24. The evaluation was performed by a well-trained examiner who was not involved in the video-based behavior analysis.

2.3. Spontaneous movement assessment

A mini-DV camcorder (SONY DCR-TRV 50) was placed in front of the monkey cage, which recorded spontaneous movements of the animals for 20 min (with nobody in the room). The video records were analyzed by a Vigie Primate video-based analysis system (View Point, Lyon, France (Chassain et al., 2001; Liu et al., 2009)). The images were digitized with a 720×480 pixel definition on 256 gray levels according to the NTSC TV standard, and the changes in pixels from one image to the next were counted every 66.67 ms.

2.4. Statistical analysis for behavior

The amount of spontaneous behavior was analyzed by a Student's *t*-test between groups (MPTP vs. normal group). A linear regression analysis was applied for a correlation analysis between the clinical rating score and counts of spontaneous movement. We further analyzed the pattern of movements in the spontaneous behaviors by repeated ANOVA. A post hoc Bonferroni analysis was used to compare among groups of monkeys with different severities of MPTP-induced parkinsonism. We considered *p*-values less than 0.05 as significant. For the repeated ANOVA, a Huynh–Feldt adjustment was applied to *p*-values when Mauchy's sphericity test was significant.

2.5. [^{11}C]-CFT-PET scan

Eight of the MPTP-treated animals (of 13) and five normal animals were used for a PET study. For the scan, the animals were placed under general anesthesia with a continuous intravenous infusion of propofol (6 mg/kg/h), and their respiration state was adjusted to normal range ($PaO_2 > 100$ mmHg, $PaCO_2 \sim 35$ mmHg) by altering the ventilation rate. The PET scan was started 2 h after achieving a physiologically stable state. After a transmission scan using a ^{68}Ge/^{68}Ga rod source for attenuation correction, a mean of 187MBq of [^{11}C]-CFT was injected at the start of a 2D dynamic PET scan lasting 60 min ($10\,s \times 18$, $30\,s \times 6$, $120\,s \times 7$, $300\,s \times 8$). The PET scan was performed on an ECAT EXACT HR+ PET scanner (Siemens-CTI, Knoxville, USA). The images were reconstructed using a filtered back projection algorithm with a Gaussian filter size of 2 mm. On different days, we obtained 3D T1-weighted magnetic resonance images (MRI) for co-registration and structural normalization for each animal (IR-FSPGR, TR = 9.4 ms, TE = 2.1 ms, TI = 600 ms) using a 3T MRI scanner (Signa LX VAH/I, GE, Milwaukee, USA).

2.6. PET data analysis

Dopamine transporter binding in the brain was quantified voxel-by-voxel as a binding potential (BP_{ND}) image, based on the simplified reference tissue model (Gunn et al., 1997). The reference region was defined in the cerebellum by delineating the boundary on a T1-weighted image that was pre-registered to the time-integrated PET image, and the time-radioactivity curve obtained in the reference region was used to calculate the binding potential image. We calculated a tracer delivery image (RI image), which was used for the next step of co-registration between PET and MRI images.

2.7. Registration across modalities (PET, MRI) and to the standard space

Before registration, non-brain structures were removed from MRI and PET images using Brain Extraction Tools in FSL (Smith, 2002). Then, for each subject, all of the MRI images were realigned to the T1 image by rigid body transformation (using FLIRT). The T1-weighted image was transformed to the standard space of a *Macaque fascicularis* brain (Hayashi et al., 2004) by affine transformation. This transformation matrix was then applied to all the MRI and PET data to be analyzed in the same standard space across modalities and individuals.

2.8. Voxel-based statistics of BP_{ND} images of [^{11}C]-CFT

The voxel-based statistics of BP_{ND} images of [^{11}C]-CFT were carried out using FEAT Version 5.98, part of FSL (FMRIB's Software Library). The following pre-statistics processing was applied to each

image: co-registration between subjects' PET-BP_{ND} images using FLIRT (FMRIB's linear image registration toolkit) followed by spatial smoothing using a Gaussian kernel of FWHM 4.0 mm. When needed, we also scaled the voxel-based maps of BP_{ND} to the global mean value in the entire brain volume. Statistical analysis was carried out using FILM without temporal filtering. For comparison between normal and MPTP-treated animals, we estimated the contrast of the group effect in BP_{ND} images. For correlation analysis of BP_{ND} images with behavioral estimations, both the qualitative rating scale (ranging from 4 to 11) and spontaneous movement scores (ranging 12–161.2) were de-meaned (i.e., subtracted by each mean value) and separately entered into the design matrices for statistical analysis. *Z*-statistic (Gaussianized *T*/*F*) images were thresholded using clusters determined by $Z > 2.3$, and were assigned a (corrected) cluster significance threshold of $p = 0.05$ (Worsley, 2001). We also performed an analysis of the regions of interest (ROI) at the putamen, caudate, ventral striatum and SN. The obtained BP_{ND} values were plotted against each group, region, and side. An ANOVA was also performed with the following factors: region, group, side, and the interaction terms. To demonstrate a correlation between BP_{ND} values and behavioral estimations, BP_{ND} values, derived from the voxel having the most significant effect in the voxel-based correlation analysis, were plotted against the behavioral estimations.

3. Results

3.1. Behavioral evaluation

The video-based analysis demonstrated that the amount of spontaneous movement was significantly less (an 88% reduction) in the MPTP-treated monkeys compared to normal monkeys [Fig. 1A; $t(20) = 5.2$, $p < 0.01$]. The parkinsonian monkeys were then divided into three groups by qualitative rating scores; mildly affected: less than 5 points ($n = 4$); moderately affected: 5–9 points ($n = 4$); severely affected: more than 10 points ($n = 5$). When analyzed by ANOVA, we found that the extent of spontaneous movement was significantly different among these three groups ($F(2,10) = 29.3$, $p < 0.001$) and post hoc multiple paired comparison disclosed a significant group difference between mildly vs. moderately affected groups ($p < 0.01$, Turkey HSD); moderately vs. severely affected ($p < 0.01$); and mildly vs. severely affected ($p < 0.001$) (Fig. 1B). The extent of movement score was negatively correlated with qualitative rating scores ($y = -12.2x + 170.1$, $R^2 = 0.792$, $t_{24} = -6.48$, $p < 0.0001$; Fig. 1C). For further investigation of the altered movement patterns in the parkinsonian monkeys, we classified the size of the movements into four levels by the amount of pixel changes (large: over 501 pixels per 66.67 ms; medium: 101–500 pixels; small: 11–100 pixels; no movement: less than 10 pixels), and the total time for each level of movements was counted for each monkey group, including normal monkeys. A repeated ANOVA (with factors of movement size, group, and interaction terms) revealed significant effects of movement size ($F(2.1, 36.2) = 71.3$, $p < 0.001$), an interaction of movement size and group ($F(6.4,36.2) = 30.3$, $p < 0.001$), but there were no significant affects of group ($F(3,17) = 0.68$, $p = 0.58$) on the amount of pixel changes; this suggests that the movement types differed by group. As shown in Fig. 2A, the period of medium- and large-sized movements decreased according to the severity of neurological symptoms in the MPTP-treated monkeys. However, the period of small movements significantly increased ($p < 0.001$) whereas the non-movement period decreased in mildly affected models of parkinsonism ($p < 0.05$) compared to those in normal monkeys. A visual rating of the video records revealed that normal monkeys exhibited large movements like climbing and jumping, while severely affected models remained almost still. In the most mildly affected animals, both resting and postural hand tremor were shown in the video, and the raw data analysis of pixel changes revealed a sequential pattern of 10–30 pixel changes with 5–6 Hz periodicity (Fig. 2B). These results suggest that video-based analysis can provide objective quantification of monkey's movements that correlate with other behaviors such as posture and balance.

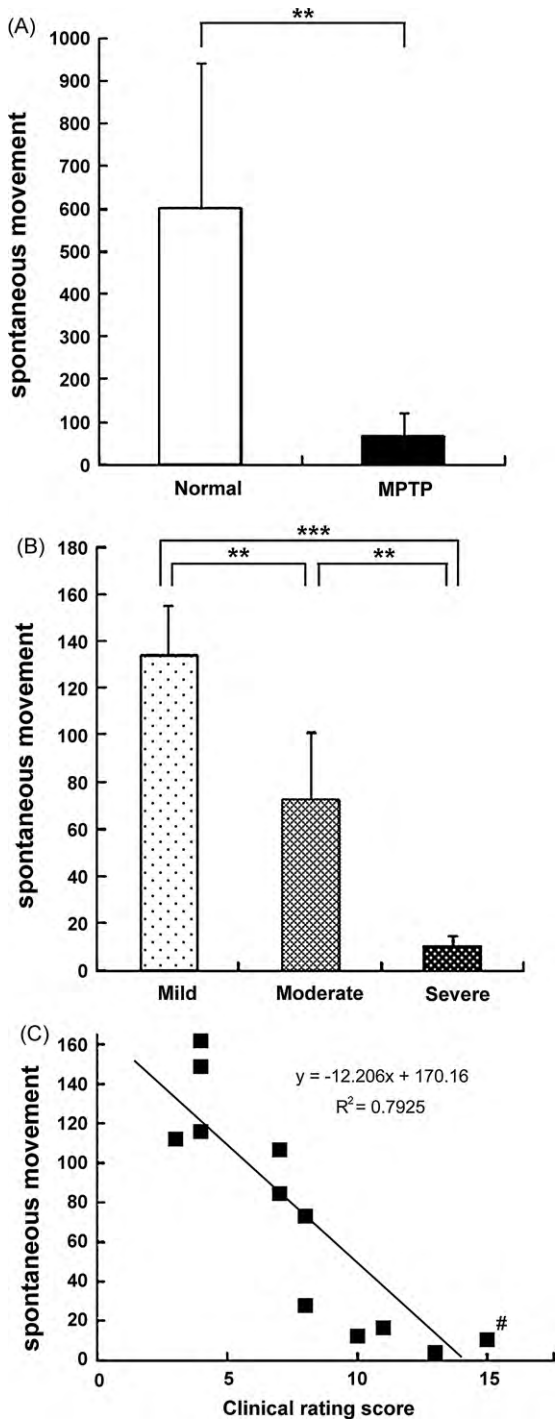

Fig. 1. Correlation between neurological score and spontaneous movement. (A) Spontaneous movement (pixel changes/66.67 ms) of normal ($n = 8$) and MPTP-treated monkeys ($n = 13$). (B) Comparison of spontaneous movement between mild ($n = 4$), moderate ($n = 4$), and severe ($n = 5$) models. Data are presented as the means ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$, Turkey HSD. (C) The plot of each sample. ($n = 13$). #Two values are too close to distinguish on the graph (score = 15, pixel change = 10.6; score = 15, pixel change = 11.0).

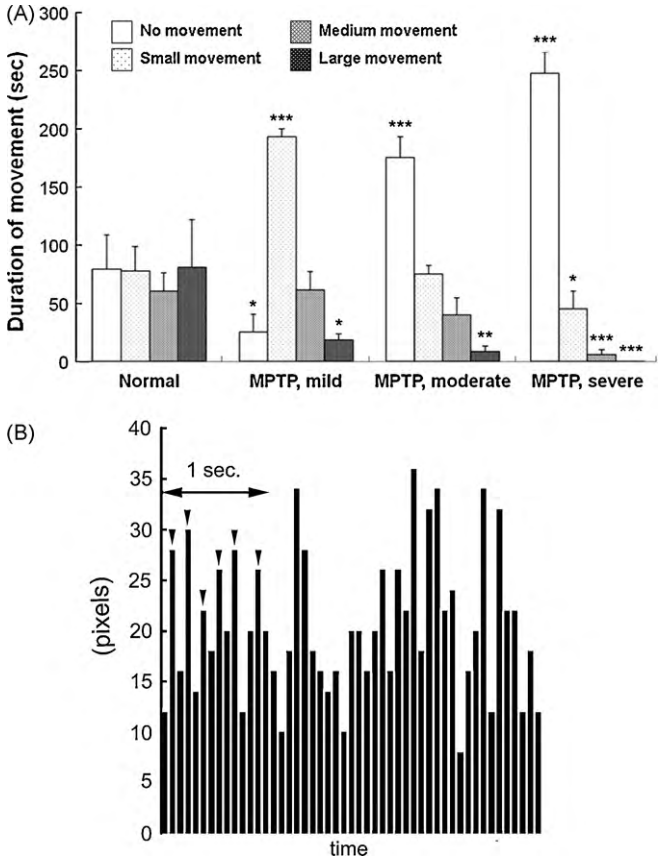

Fig. 2. Assessment of movement patterns in MPTP-treated monkeys. (A) Total duration of movement in normal and model monkeys. Data are presented as the means ± SD. Each value was compared with the one of normal monkeys. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (B) Representative pixel changes per each time unit (66.67 ms) in the mildly affected monkey.

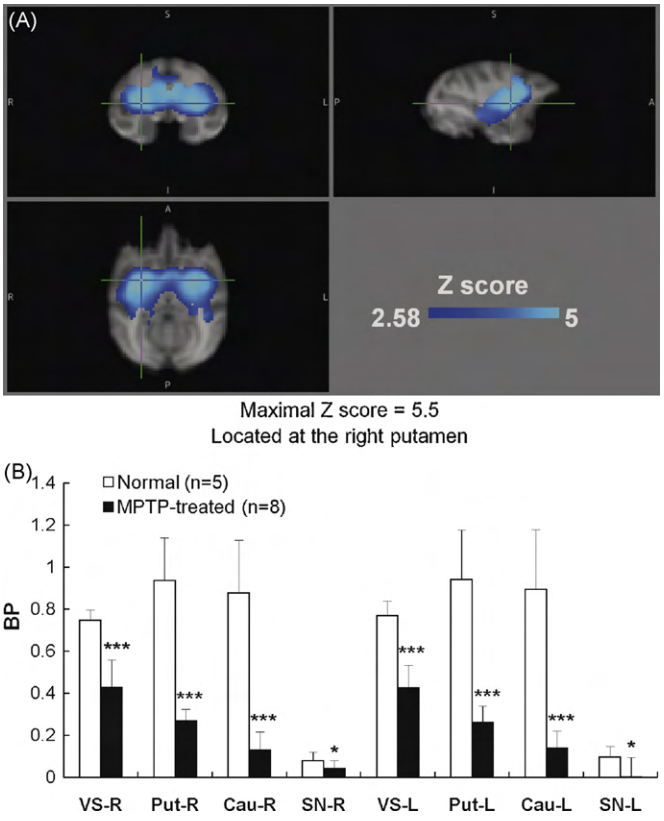

Fig. 3. Group comparison of [^{11}C]-CFT BP_{ND} revealed by PET. (A) Voxel-based statistical results of group comparison between normal and MPTP-treated monkeys. Significant cluster ($Z > 2.3$, corrected $p < 0.05$) was found in the contrast of (normal > MPTP-treated), indicating decrease of CFT BP_{ND}. The maximal Z score was found in the right putamen (crosshair). (B) Bar plot of BP_{ND} values obtained by region of interest (ROI) analysis. Simple group comparison of BP_{ND} values for each region showed significant decrease in BP_{ND} in the putamen (Put), caudate (Cau), ventral striatum (VS) and substantia nigra (SN) for both side (L, left; R, right). $^{*}p < 0.05$, $^{***}p < 0.001$.

3.2. DAT function

In a PET analysis for DAT function, we first performed group comparisons between normal and MPTP-treated animals. A notably high binding of [^{11}C]-CFT was observed in the striatum in normal monkeys; this binding was much reduced in the MPTP-treated animals, particularly in the dorsal striatum. Voxel-based statistics for group comparison without performing the multiplicative mean voxel-value normalization revealed that the binding potential (BP) significantly decreased in a cluster involving the putamen and caudate nucleus, but mostly spared the rostro-ventral part of the striatum (Fig. 3A). The BP_{ND} values in the ROI analysis are shown in Fig. 3B. A repeated ANOVA revealed significant effects of group ($F(1,11) = 80.3$, $p < 0.001$), the interaction of regions and groups ($F(2,22) = 16.2$, $p < 0.001$) and a marginal effect of regions ($F(1.3,14.3) = 3.28$, Huyn–Feldt adjusted, $p = 0.083$), but no significant effect of side ($F(1,11) = 0.31$, $p = 0.6$) on the BP values. In fact, the subsequent comparison of the group (normal vs. MPTP) for each region (including left and right side) disclosed a smaller decrease of [^{11}C]-CFT BP_{ND} in the ventral striatum (−44% difference in mean values, $T(24) = 8.36$, $p < 0.001$) than in the dorsal striatum (caudate: −85%, $T(24) = 11.1$, $p < 0.001$, putamen: −72%, $T(24) = 12.2$, $p < 0.001$) (Fig. 3B).

We performed a voxel-based correlation analysis between [^{11}C]-CFT BP_{ND} and spontaneous movements as well as qualitative rating scores in the MPTP-treated monkeys. We note that this was an unbiased analysis, i.e., a voxel-based comparison throughout the entire brain volume. Without multiplicative mean BP_{ND} value normalization (i.e., quantitative BP_{ND} images were used “as is” in the statistical analysis), there were no significant voxels or clusters in the voxel-based correlation analysis, either for qualitative rating score or spontaneous movement. We then applied multiplicative mean voxel-value normalization before the statistical analysis and found a significant cluster located in the midbrain for both qualitative rating score (Fig. 4A) and spontaneous movement (Fig. 5A); however, there was no significant difference in the striatal voxels as expected. With the BP_{ND} values plotted, we found a negative correlation in the midbrain for qualitative rating score (Fig. 4B) and a positive correlation for spontaneous movement (Fig. 5B). These results suggest that neurological symptoms of MPTP-treated monkeys are more related to the DAT density in the midbrain than in the striatum.

4. Discussion

We showed that a video-based analysis system can detect a difference in spontaneous movements between normal and MPTP-treated monkeys. Moreover, we clearly showed a significant correlation between the quantity of spontaneous movements and the severity of qualitative rating scores. These results are consistent with previous studies which found a significant correlation of rating scale and the residual dopaminergic neurons in post-mortem evaluation (Elsworth et al., 2000). Other studies in clinical patients also found similar relationships between a DAT binding ligand and a clinical rating scale (UPDRS) in PD patients (Marek et al., 2001; Seibyl et al., 1995).

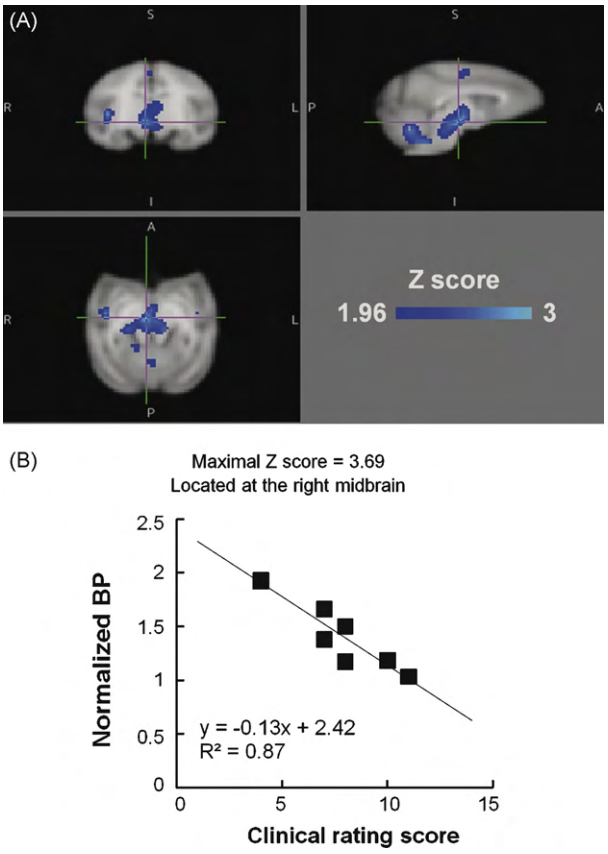

Fig. 4. Correlation analysis of [^{11}C]-CFT BP_{ND} with qualitative rating score. (A) Voxel-based statistical results of correlation with qualitative rating score. Significant cluster ($Z > 2.3$, corrected $p < 0.05$) was found in the contrast of negative correlation with qualitative rating score. The maximal Z score was found in the right midbrain, close to the right substantia nigra (crosshair). (B) The plot of the normalized BP_{ND} values at the right midbrain voxel with the maximal Z score in A.

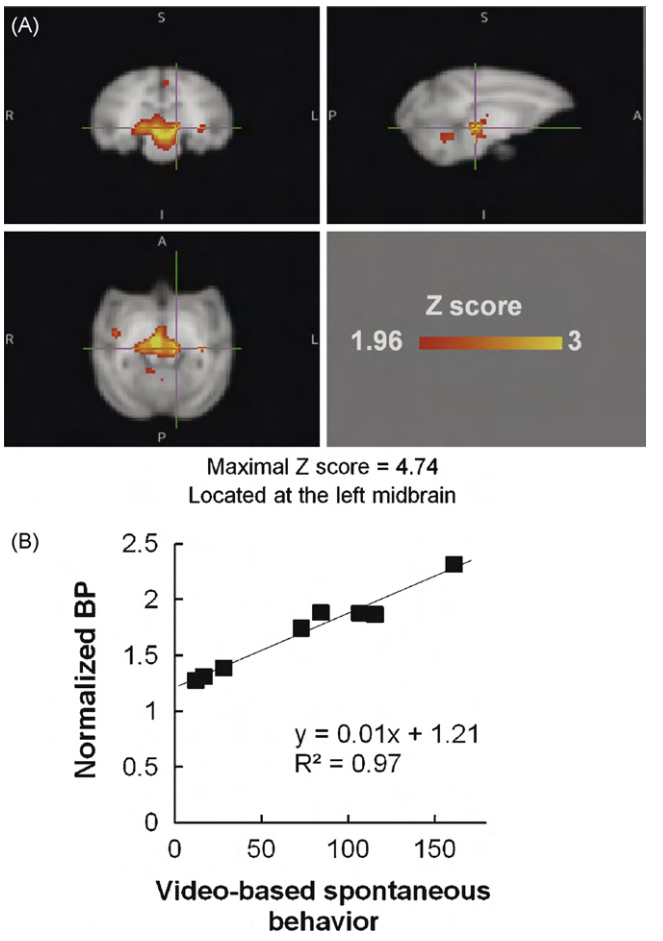

Fig. 5. Correlation analysis of [^{11}C]-CFT BP_{ND} with spontaneous movement. (A) Voxel-based statistical results of correlation with qualitative rating score. Significant cluster ($Z > 2.3$, corrected $p < 0.05$) was found in the contrast of positive correlation with video-based spontaneous behavior. The maximal Z score was located in the left midbrain, close to the left substantia nigra (crosshair). (B) The plot of the normalized BP_{ND} values at the left midbrain voxel with the maximal Z score in A.

It has recently been reported that bradykinesia and rigidity were closely related, and that these items showed weaker correlations with other items such as freezing and tremor (van Rooden et al., 2009). This suggests that the component variables in a qualitative rating scale are not independent. Therefore, the correlation in this study seems reasonable. In fact, other video-based analyses of an acute phase (3–4 weeks) of MPTP-treatment with squirrel monkeys (Togasaki et al., 2005) or hemiparkinsonian cynomolgus monkeys (Liu et al., 2009) demonstrated a significant correlation between spontaneous movements and qualitative rating scores. In this study, we showed this correlation with a more chronic (more than 12 weeks) phase of a cynomolgus monkey model by systemic MPTP-treatment. Since the qualitative rating scores can be observer-dependent and may not be parametric evaluations if not properly categorized (see below), the video-based analysis can be a reliable tool for the objective quantification of motor function.

The qualitative rating scale used in the present study has potential drawbacks. It principally includes items that may not necessarily be specific to PD symptoms (alertness, head checking movement), and it would tend to exaggerate the behavioral change due to the overlap of similar items (for example, balance and walking). Because of the difficulty in verbal communication, there seem to be no perfect qualitative rating scale for monkey behaviors (Imbert et al., 2000), but our qualitative ratings scores may need to be optimized to increase the specificity and sensitivity to behavioral symptoms of PD model.

In addition to a simple comparison between normal and MPTP-treated monkeys, we investigated the alteration of movement patterns between the mildly, moderately, and severely affected monkeys and found that mildly affected monkeys exhibited more small movements than normal monkeys. This may be due to an increase in resting and postural hand tremor in mildly affected animals. In fact, both resting and postural hand tremor, apparent on the video monitoring, exhibited an amplitude of 10–30 pixels and a frequency of 6 Hz. We would like to emphasize that the video-based analysis is able to distinguish tremor and count its frequency. In human patients, a large body of clinical data indicates that tremor is predominantly observed in early stage or mildly affected patients (Kang et al., 2005) and later with the progression of the disease, the increased akinesis or rigidity conceals the tremors. Consistent with this idea, it is often reported that appropriate administration of anti-parkinsonian medication leads to the reappearance of resting tremor in PD patients. In contrast, there is another argument that such tremor-dominant models are pathophysiologically different from other models in terms of their vulnerability to MPTP toxicity, since the disease of tremor-dominant PD is known to have a relatively benign course compared to the cases with marked bradykinesia (Jankovic and Kapadia, 2001). These ideas should be further explored by future studies, including, for example, the repeated assessment of behaviors during the progression of symptoms in the course of a MPTP-poisoning regimen or during long-term follow up.

Our PET study showed an extensive decrease of [^{11}C]-CFT BP_{ND} in the striatum of the MPTP-treated monkeys, particularly in the dorsal region. This is consistent with previous studies on MPTP-treated monkeys (Brownell et al., 2003) and human PD patients (Nurmi et al., 2003; Rinne et al., 2001). The preferential depletion of DAT in the dorsal, rather than ventral striatum is also consistent with previous studies using PET (Doudet et al., 2006) and pathological assessment (Chiueh et al., 1985). More importantly, in our unbiased analysis through the whole brain of MPTP-treated animals, we found that [^{11}C]-CFT BP_{ND} in the midbrain was significantly correlated with both of our behavioral assessments, suggesting that DA transmission in the midbrain is also involved in the deterioration of motor behaviors but shows a different correlation from that in the striatum. This finding is unexpected since a number of clinical studies in PD patients have shown a correlation of the degree of symptoms with the residual DA in the striatum. Since the current model animals were all in the chronic stage after systemic injection of MPTP, it may reflect the pathophysiology of advanced PD, in which compensatory changes occur in response to the degeneration of DA neurons.

We hypothesize that the midbrain DAT and DA might have a functional, compensatory role disparate from that in the striatum in MPTP-treated animals, when their striatal DA terminals were severely affected. The current animals all showed severely depleted DAT in the striatum, particularly in the motor-related area (i.e., putamen) of the striatum (~20% of normal animals). In addition, the current findings were obtained by the normalization of the global mean BP_{ND} value, which removes variations in the residual CFT BP_{ND} in the striatum from the BP_{ND} values. In fact, the global mean BP_{ND} values obtained here in MPTP-treated animals showed a highly significant correlation with the mean striatal BP_{ND} values (Peason's correlation coefficient $r = 0.78$, $t_6 = 3.05$, $p < 0.05$), which were derived from the ROI covering the bilateral striatal areas. Thus, the results of the significant correlation between the midbrain BP_{ND} and the behavioral assessments should depend on the level of DAT depletion in the striatum.

Accumulated evidence has suggested that somatodendritic DA release in SN has a distinct function other than nigro-striatal DA release (Cheramy et al., 1981; Cobb and Abercrombie, 2002; Robertson, 1992; Timmerman and Abercrombie, 1996). Observations in 6-hydroxydopamine (6-OHDA)-treated rats showed that CFT binding in the striatum reflected the number of nigral DA neurons more directly than that in the SN (Forsback et al., 2004), suggesting that DAT in the SN might be associated with a compensatory response to DA cell injuries. In another rodent study, the effect of DA depletion on motor performance was more evident when toxins were locally applied to SN than when applied to the striatum (Andersson et al., 2006). Taken together, our results suggest that changes in somatodendritic DAT in SN have a pivotal plastic function in motor performance, and that this function is distinct from the nigro-striatal DA system. Dendrites from the DA neurons located in SN pars compacta (SNc) extend throughout SN pars reticulata (SNr; (Prensa and Parent, 2001), and their release of DA has been shown to regulate the activity of the DA neurons themselves, the release of GABA from afferent fibers in SNr, and then the activity of its efferent projections (Cheramy et al., 1981; Robertson, 1992). Thus, our observations also support the pathophysiology of PD, where GABAergic output from SNr/internal segment of GP is a key controller of parkinsonian symptoms (DeLong, 1990). As to the functional role of DA transmission in the midbrain, future studies including pathological studies and other imaging modalities that can evaluate neuronal activity such as [^{18}F]-FDG PET or post-synaptic D1/D2 dopamine receptors should be performed.

In conclusion, in the current study with PD models in non-human primates, we showed that video-based analysis can be a reliable tool for an objective and quantitative evaluation of motor function. We also demonstrated that DAT function in the midbrain, not in the striatum, was correlated to the motor behaviors of the MPTP-treated monkeys, suggesting the functional segregation of midbrain DA release from striatal one.

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